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TITLE: Detection of Prostate Cancer Progression by Serum DNA Integrity

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14. ABSTRACT
The main objective/hypothesis for this proposal is that additional diagnostic and prognostic insight can be obtained through analysis of tumor-related DNA integrity and methylation in patient serum. Additionally, DNA biomarkers will be assessed for their usefulness in predicting early disease recurrence and progression in patients during PCa treatment. The scope of the studies is to optimize and validate Alu, LINE1 and uLINE1 assays for assessment of prostate cancer (PCa) patient serum targeted for clinical utility. For the first year, we have put in place specimen procurement and processing protocols and optimized assays for Alu qRT and LINE1 qRT. The uLINE1 AQAMA (absolute quantitative allele methylation assay) assay is also near optimal. We have utilized prostate cell lines and tumor specimens for the initial assay optimization and validation. In the coming year,more PCA patients will be accrued as well as further accrual of serum at defined treatment intervals. Assays for the markers will be carried out once we have obtained statistically meaningful numbers of serum sets of PCa patients in treatment. We are also continuing our efforts in discovering additional clinically relevant DNA biomarkers.

15. SUBJECT TERMS
Circulating DNA, serum, prostate cancer, methylation, PCR

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INTRODUCTION

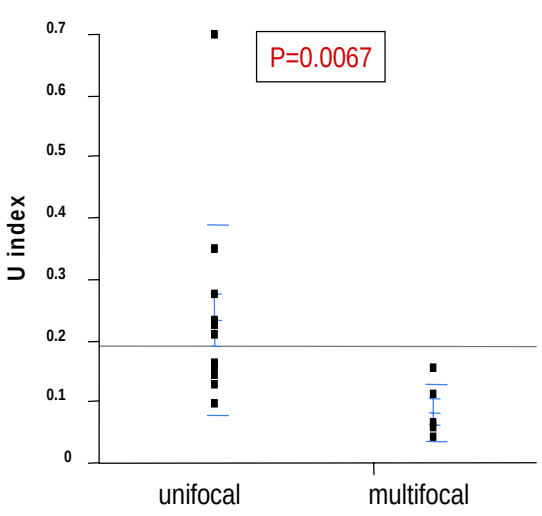
The main hypothesis of the proposal is that the detection of serum circulating tumor-related DNA marker(s) are surrogate genetic indicators of primary prostate cancer (PCa) tumor status and can be used to facilitate diagnosis, prognosis, predict treatment response, and aid in the surveillance of early disease recurrence. In the program years, our efforts will specifically focus on 1) Assessment of the *Alu* DNA integrity marker in PCa patients' serum using a quantitative direct assay to determine its clinical utility; 2) Assessment of LINE1 DNA integrity marker and uLINE1 marker in PCa patients' serum using quantitative assays to determine their clinical utility; and 3) Determine the combined clinical utility of the three circulating serum tumor-related DNA markers in monitoring patients' response to treatment. The long-term goal is to validate the clinical utility of these markers.

BODY

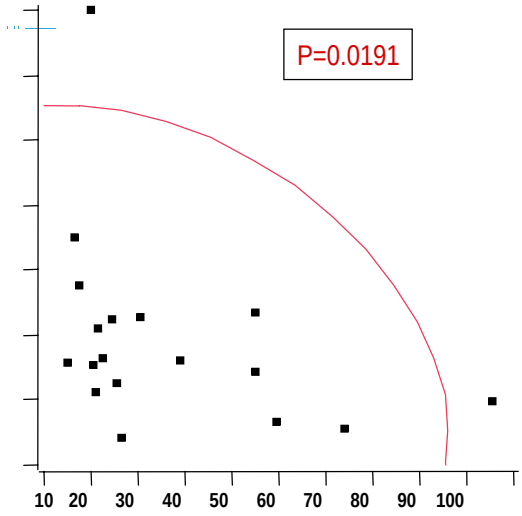
In this first year of the proposal period, we began accruing PCa patients and normal donor serum for the study with an IRB approved protocol. As outlined in Task 1, Samples are being collected, coded, logged into a database, processed for serum and quality assured. Task 1 will be ongoing until we reach our proposed sample numbers.

Optimization of Alu and LINE1 assays are currently underway. The use of PCa cell lines facilitated the progress of the task. By using paraffin-embedded (PE) PCa tissue samples as clinical samples, we were able to further validate our assays. We have demonstrated proficiency in DNA extraction from paraffin-embedded tissues which were first micro-dissected using LCM. Because of the multifocal nature of the tumor, micro-dissection is necessary to isolate the cancer cells for validation of these markers as tumor markers.

We have devoted major efforts in developing the uLINE1 AQAMA assay. The protocol involved designing and testing specific primer sequences for methylated (LINE 1) and unmethylated (uLINE1) LINE1. In a pilot study, 18 prostate cancer PE tissues with matched adjacent normal tissues were assessed with the assay. Although the specimen numbers are limited, we observed that tumors tend to show higher uLINE1 index compared with normal tissue. Additionally, unifocal cancer showed significantly high U index compared with multifocal cancer (p=0.0067) (Figure A). Tumor U index is significantly correlated with prostate volume (p=0.0191)(Figure B).

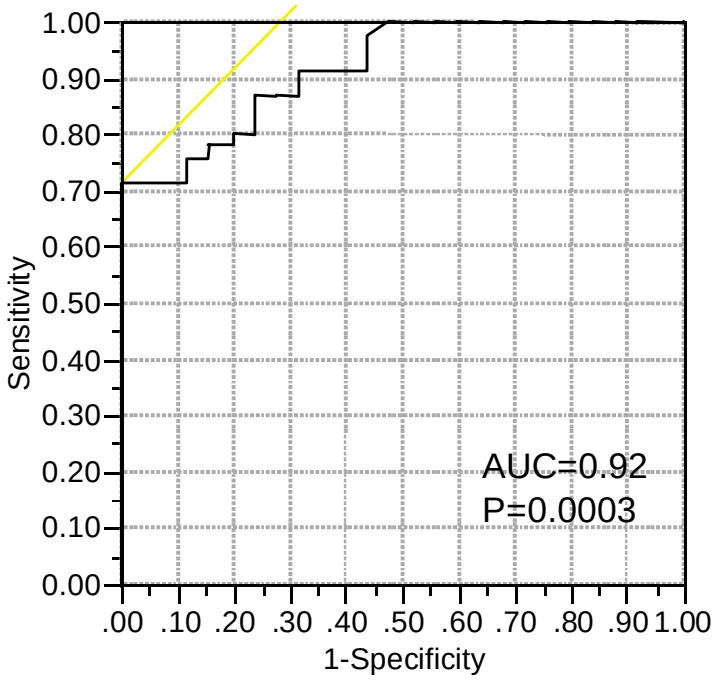


A. multifocality



B. Prostate volume, cc

In patient serum samples, we have worked on optimizing the assay. We were able to use samples from a small patient population and normal donors to establish the sensitivity and specificity of the LINE1 assay (Figure C).



C. ROC Curve for uLINE1 copies in serum DNA

So far, with the limited samples we have tested, we were able to confirm LINE1 is highly methylated in normal male donor serum and unmethylated in prostate cancer patients.

We plan to continue our efforts and optimize these assays to begin assessing patients serum in the coming year. We will accrue patients with BPH and prostatitis as well as age matched normal male donors. Refer to the grant tasks itemization and list for our future direction.

KEY RESEARCH ACCOMPLISHMENTS

1. PCa patient accrual for serum.
2. Normal healthy donor serum accrual for serum.
3. Database constructed and utilized to keep track of samples.
4. Clinical data recorded and collected in the database.
5. Blood is processed for serum; DNA is extracted and quantified
6. LINE1 methylation biomarkers in serum are detected.
7. LINE1 methylation biomarkers assessed and optimized for detection in serum.
8. LINE1 methylation biomarkers optimized for specificity and sensitivity.

REPORTABLE OUTCOMES

No reportable outcomes have occurred during this time period.

CONCLUSIONS

The planned studies are being conducted according to the approved schedule as delineated in the protocol.

REFERENCES

None.

LIST OF PERSONNEL

Name	Institution	Role
Dave S.B. Hoon, MSc, PhD	John Wayne Cancer Institute at Saint John's Health Center	Principal Investigator
Frederick R. Singer, MD	John Wayne Cancer Institute at Saint John's Health Center	Co-Investigator
David Elashoff, PhD	University of California, Los Angeles	Co-Investigator
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APPENDICES

None.